CLAIMS

WHAT IS CLAIMED IS:

1. A differentiated cell population in an *in vitro* culture obtained by differentiating primate pluripotent stem cells, wherein at least 60% of the differentiated cells are dopaminergic neurons.

2. The cell population of claim 1, wherein the primate pluripotent stem cells are human embryonic stem cells.

3. A differentiated cell population in an *in vitro* culture obtained by differentiating primate pluripotent stem cells, wherein at least 60% of the differentiated cells express tyrosine hydroxylase.

4. The cell population of claim 3, wherein the primate pluripotent stem cells are human embryonic stem cells.

5. A method of generating a differentiated neural cell population from primate pluripotent stem cells comprising the following steps:
    (a) expanding a culture of primate pluripotent stem cells;
    (b) culturing the pluripotent stem cells to select for neuroprogenitor cells that are positive for nestin;
    (c) sorting the nestin-positive neuroprogenitor cells for enrichment of NCAM-positive cells;
    (d) differentiating the nestin-positive, NCAM-positive cells to generate a differentiated neural cell population by culturing the cells in a differentiation media which comprises TGF-β3 or interleukin-1β or both.

6. The method of claim 5, wherein the pluripotent stem cells were derived using a laser ablation technique.

7. The method of claim 5, wherein the pluripotent stem cells are human embryonic stem cells.

8. The method of claim 7, wherein the human embryonic stem cells were derived using a laser ablation technique.

9. The method of claim 5, wherein the differentiated neural cell population comprises at least about 60% dopaminergic neurons.

10. The method of claim 5, wherein the differentiated neural cell population comprises at least about 30% serotonergic neurons.

11. The method of claim 5, wherein the differentiated neural cell population comprises at least about 25% oligodendrocytes.

12. The method of claim 5, further comprising culturing the pluripotent stem cells of step (b) to form embryoid bodies.

13. The method of claim 12, wherein the embryoid bodies are cultured to select for neuroprogenitor cells that are positive for nestin.

14. The method of claim 5, wherein the neuroprogenitor cells that are positive for nestin are selected by culturing the pluripotent stem cells in serum-free medium.

15. The method of claim 14, wherein the serum-free medium is ITSFn serum-free defined medium.

16. The method of claim 14, wherein the serum-free medium comprises one or more soluble factors selected from the group consisting of insulin, sodium selenite, transferrin, and fibronectin.

17. The method of claim 16, wherein the serum-free medium comprises insulin, sodium selenite, transferrin, and fibronectin.

18. The method of claim 13, wherein the neuroprogenitor cells that are positive for nestin are selected by culturing the embryoid bodies in serum-free medium.

19. The method of claim 18, wherein the serum-free medium is ITSFn serum-free defined medium.

20. The method of claim 18, wherein the serum-free medium comprises one or more soluble factors selected from the group consisting of insulin, sodium selenite, basic fibroblast growth factor, transferrin, and fibronectin.

21. The method of claim 20, wherein the serum-free medium comprises insulin, sodium selenite, transferrin, and fibronectin.

22. The method of claim 21, wherein the neuroprogenitor cells comprise at least about 95% nestin-positive cells.

23. The method of claim 5, wherein the nestin-positive neuroprogenitor cells of step (c) are sorted to enrich for NCAM-positive cells by Magnetic Cell Sorting (MACS).

24. The method of claim 23, wherein the nestin-positive neuroprogenitor cells comprise at least about 50-60% NCAM-positive cells.

25. The method of claim 5, further comprising expanding the nestin-positive, NCAM-positive neuroprogenitor cells of step (c) in expansion medium.

26. The method of claim 25, wherein the expansion medium comprises one or more soluble factors selected from the group consisting of insulin, sodium selenite, transferrin, laminin, putrescine, progesterone, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), sonic hedgehog (SHH), fibroblast growth factor-8 (FGF-8), and brain derived neurotropic factor (BDNF).

27. The method of claim 26, wherein the cells are grown in the expansion medium for 6-10 days.

28. The method of claim 26, wherein the cells are cultured and serially passed for one or more population doublings.

29. The method of claim 26, wherein the cells are cryopreserved in liquid nitrogen.

30. The method of claim 5, wherein the differentiation media comprises Neurobasal medium supplemented with fetal calf serum, B27, ascorbic acid, and N-acetyl cysteine.

31. The method of claim 5, wherein the differentiation media further comprises one or more differentiation agents selected from the group consisting of ascorbic acid, N-acetyl, cysteine glial cell line derived neurotropic factor (GDNF), dibutyrl-cyclic AMP (db-cAMP), brain derived neurotropic factor (BDNF), neuturin, sonic hedgehog protein (SHH), and fibroblast growth factor-8 (FGF-8)

32. The method of claim 5, wherein the nestin-positive, NCAM-positive cells are grown in differentiation media for 30-50 days.

33. A method of generating dopaminergic neurons from neuroprogenitor cells, comprising enriching the neuroprogenitor cells for cells that are positive for nestin, and differentiating the nestin-positive cells to generate dopaminergic neurons by culturing the cells in the presence of TGF-β3 or interleukin-1β or both.

34. The method of claim 33, wherein at least about 40% of the nestin-positive cells differentiate into dopaminergic neurons.

35. The method of claim 33, further comprising enriching the neuroprogenitor cells for cells that are positive for NCAM.

36. The method of claim 34, wherein the nestin-positive, NCAM-positive cells are differentiated to generate dopaminergic neurons.

37. The method of claim 36, wherein at least about 60% of the nestin-positive, NCAM-positive cells differentiate into dopaminergic neurons.

38. A method of generating serotonergic neurons from neuroprogenitor cells, comprising enriching the neuroprogenitor cells for cells that are positive for nestin and NCAM, and differentiating the nestin-positive, NCAM-positive cells to generate serotonergic neurons by culturing the cells in the presence of TGF-β3 or interleukin-1β or both.

39. The method of claim 38, wherein at least about 30% of the nestin-positive, NCAM-positive cells differentiate into serotonergic neurons.

40. A method of treating a patient with a neurodegenerative disorder or neuronal disease comprising the following steps:

(a) expanding a culture of primate pluripotent stem cells;

(b) culturing the pluripotent stem cells to select for neuroprogenitor cells that are positive for nestin;

(c) sorting the nestin-positive neuroprogenitor cells for enrichment of NCAM-positive cells;

(d) differentiating the nestin-positive, NCAM-positive cells to generate a differentiated neural cell population by culturing the cells in a differentiation media which comprises TGF-β3 or interleukin-1β or both;

(e) transplanting a therapeutically effective amount of the differentiated neural cell population into the central nervous system of the patient.

41. The method of claim 40, wherein the primate pluripotent stem cells are human embryonic stem cells.

42. The method of claim 40, further comprising isolating dopaminergic neurons from the differentiated neural cell population and administering the dopaminergic neurons to the patient.

43. The method of claim 40, wherein the neurodegenerative disorder or neuronal disease is selected from the group consisting of Parkinson's disease, Alzheimer's disease, Huntington's disease, spinal cord injury, amyotrophic lateral sclerosis (ALS), epilepsy, stroke, and ischemia.

44. The method of claim 40, wherein the differentiated neural cell population is transplanted into the brain of the patient.